

	CEC (1991)	Gettings et al. (1991)	Bagley et al. (1992)	Gettings et al. (1994)	Vinardell and Macián (1994)
Hen Strain	Lohmann Selected Leghorn	Lohmann's Selected White Leghorn LSL	White Leghorn	White Leghorn	Leghorn SA31
Egg Criteria for Use	Weight range of eggs between 50 g and 60 g	Not Noted	Weight range of eggs between 50 g and 60 g	Not Noted	Not Noted
Egg Storage (Prior to use)	Not Noted	Not Noted	Not Noted	Not Noted	Not Noted
Incubation Temperature (°C)	37.5 ± 0.5	Not Noted	37.5 ± 0.5	38	Not Noted
Relative Humidity (%)	62.5 ± 7.5	Not Noted	62.5 ± 7.5	60	Not Noted
Egg Rotation?	Yes	Not Noted	Yes	Yes	Not Noted
Checking Egg Viability	Eggs candled on Day 5 and every day thereafter; non-viable embryos removed	Not Noted	Not Noted	Candled on Day 9 and non-viable embryos removed	Not Noted
Incubation Period	10 Days	10 Days	10 Days	9 Days	10 Days
Procedure for Opening Egg	Egg shell was scratched around the air chamber with a rotating dentist saw blade and then pared off. The inner shell was removed and the CAM was layed open.	Not Noted	Egg shell around the air pocket was removed with a dental rotary saw	Eggshell was scratched around the air cells and a small aperture was opened.	Egg shell was scratched around the air cell by a dentist's rotary saw and then pared off. After removal of the inner membrane, the CAM was exposed.
Manipulation of CAM	Not Noted	Not Noted	Not Noted	Not Noted	Not Noted

	CEC (1991)	Gettings et al. (1991)	Bagley et al. (1992)	Gettings et al. (1994)	Vinardell and Macián (1994)
Investigator Defined Test Substance Classes	Chemicals from various chemical classes	Hydro-alcoholic formulations	Chemicals from various chemical classes, commercial products, and personal care products	Oil/water -based personal care formulations	Commercial disinfectants
Total Test Substances Evaluated	21	10	32	18	6
Test Substance Quantity or Volume	0.3 mL or 0.1 g	Not Noted	0.3 mL or 0.1 g	0.3 mL	0.3 mL
Test Substance Concentrations Tested	0.1% to 100%	Not Noted	Tested solutions at concentrations that were 10% of those tested <i>in vivo</i>	3 concentrations tested: threshold concentration, 10% solution, undiluted	Diluted or undiluted
Application of Solids to CAM	Placed directly on CAM	Not Noted	All tested substances appear to be solubilized	All tested substances appear to be solubilized	All tested substances appear to be solubilized
Preferred Solvent	Not Noted	Not Noted	Not Noted	Not Noted	Distilled water
Rinse after Test Substance Application?	Yes, for solids. 20 seconds after application of test substance, rinsed with 5 mL warm water or saline solution	Not Noted	20 seconds after test substance applied rinsed with 5 mL warm water	Not Noted	Not Noted
Evaluation Period	Up to 300 seconds after test substance applied	Not Noted	At 0.5, 2, and 5 minutes after test substance applied	Up to 300 seconds after test substance applied	Up to 300 seconds after test substance applied
Controls and Test Standards	Not Noted	Not Noted	Vehicle	Not Noted	0.1 M NaOH, 1% SDS, 0.9% NaCl, distilled water
Number of Control Eggs	Not Noted	Not Noted	2 eggs	Not Noted	2 eggs per substance
Replicate Eggs	Minimum 6 eggs	Not Noted	4 eggs	3 eggs per concentration tested	6 eggs
Number of Replicate Experiments	Not Noted	Not Noted	Not Noted	2	Not Noted
Endpoints Assessed	Hemorrhage, lysis, and coagulation	Hemorrhage, vascular lysis, coagulation	Hyperemia, hemorrhage, coagulation	Hemorrhage, coagulation, lysis	Hemorrhage, vasoconstriction, coagulation
Endpoint Evaluation	The starting second that each of the three endpoints is observed is recorded.	The starting second that each of the three endpoints is observed is recorded.	Numerical time-dependent scores for three endpoints.	Seconds for the three endpoints recorded (see Kalweit, 1990)	The starting second that each of the endpoints is observed is recorded

	CEC (1991)	Gettings et al. (1991)	Bagley et al. (1992)	Gettings et al. (1994)	Vinardell and Macián (1994)
Analysis Method	Irritation Index is calculated using the formula: $(301 - \text{sec H})/300 * 5 + (301 - \text{sec L})/300 * 7 = (301 - \text{sec C}) * 9$; where H=Hemorrhage, L=Lysis; C=Coagulation; sec=starting second	Two different analyses were used. Both calculated an irritation index using time (seconds) of appearance of hemorrhage vascular lysis, or coagulation. The differences between the two methods was in the particular calculations used.	Scores are totaled to give a single value (maximum of 21). Mean value of 4 eggs calculated for final value.	Calculation of an Irritation Score for each egg. Mean value of individual Irritation Scores calculated.	Irritancy Potential calculated using the formula: $(301 - \text{sec H})/300 * 5 + (301 - \text{sec v})/300 * 7 = (301 - \text{sec C}) * 9$; where H=Hemorrhage, V=vasoconstriction; C=Coagulation of protein or blood; sec=starting second
Classification Scheme	Not Noted	Not Noted	Not Noted	Practically None: 0-0.9; Slight Irritation: 1-4.9; Moderate Irritation: 5-9.9; Strong Irritation: 10-21	Practically None: 0-0.9; Slight Irritation: 1-4.9; Moderate Irritation: 5-8.9; Strong Irritation: 9-21
GLP Compliance?	Not Noted	Not Noted	Not Noted	Not Noted	Not Noted
Notes		Cites Luepke (1985) as basis for protocol used	Cites Luepke and Kemper (1986) as basis for protocol	Cites Luepke (1985) as basis for scoring scheme	Cites Ergatt/Frame Data Bank (1990) as basis for protocol and analysis method
				To study the effects of slow acting materials, CAM scored 15 and 30 minutes after application on range from 0 (no reactions) to 3 (strong reactions)	

	Balls et al. (1995) (Non-Transparent Substances)	Balls et al. (1995) (Transparent Substances)	Kojima et al. (1995)	Gettings et al. (1996) (HET-CAM I, II)	Gettings et al. (1996) (HET-CAM III)
Hen Strain	Not Noted	Not Noted	Not Noted	Lohmann's Selected White Leghorn	White Leghorn
Egg Criteria for Use	Not Noted	Not Noted	Not Noted	Not Noted	Not Noted
Egg Storage (Prior to use)	Not Noted	Not Noted	Not Noted	Not Noted	Not Noted
Incubation Temperature (°C)	Not Noted	Not Noted	37.6	Not Noted	38 ± 0.5
Relative Humidity (%)	Not Noted	Not Noted	about 70	Not Noted	60 ± 5
Egg Rotation?	Not Noted	Not Noted	Yes, once per hour	Not Noted	Yes
Checking Egg Viability	Not Noted	Not Noted	Not Noted	Not Noted	Candled on Day 9 and returned to incubator in vertical position
Incubation Period	9 Days	9 Days	10 Days	10 Days	10 Days
Procedure for Opening Egg	Not Noted	Not Noted	A portion of the egg shell was removed and a drop of water was placed onto the shell membrane to avoid bleeding. Then the CAM was exposed with forceps.	Not Noted	Shell and inner shell membrane were removed around the area defined by the air cell
Manipulation of CAM	Not Noted	Not Noted	Not Noted	Not Noted	Not Noted

	Balls et al. (1995) (Non-Transparent Substances)	Balls et al. (1995) (Transparent Substances)	Kojima et al. (1995)	Gettings et al. (1996) (HET-CAM I, II)	Gettings et al. (1996) (HET-CAM III)
Investigator Defined Test Substance Classes	Chemicals selected from the ECETOC database (acids, acyl halides, alcohols, aldehyde, alkalis, esters, heterocyclics, hydrocarbons, inorganic chemicals, ketones, organophosphates, pesticides, surfactants, misc.)	Chemicals selected from the ECETOC database (acids, acyl halides, alcohols, aldehyde, alkalis, esters, heterocyclics, hydrocarbons, inorganic chemicals, ketones, organophosphates, pesticides, surfactants, misc.)	Surfactants, solvents, formaldehyde	Surfactant-based personal care formulations	Surfactant-based personal care formulations
Total Test Substances Evaluated	59	59	24	25	25
Test Substance Quantity or Volume	Not Noted	Not Noted	0.2 mL	0.3 mL	0.1 mL
Test Substance Concentrations Tested	Not Noted	Not Noted	10% solution	3 concentrations tested: threshold concentration, 10% solution, undiluted	10% solution
Application of Solids to CAM	Not Noted	Not Noted	All tested substances appear to be solubilized	All test substances appear to be in liquid form	All test substances appear to be in liquid form
Preferred Solvent	Not Noted	Not Noted	Not Noted	Not Noted	Not Noted
Rinse after Test Substance Application?	After 3 minute exposure	Not Noted	Yes, after 20 seconds test substance was rinsed with water	Not Noted	After 20 seconds, with a saline rinse
Evaluation Period	Within 30 seconds of rinsing	Up to 300 seconds after test substance applied	At 0.5, 2, and 5 minutes after test substance rinsed	Up to 300 seconds after test substance applied	At 0.5, 2, and 5 minutes after test substance rinsed
Controls and Test Standards	5% Texapon AV (internal reference standard)	5% Texapon AV (internal reference standard)	Not Noted	Not Noted	Not Noted
Number of Control Eggs	Not Noted	Not Noted	Not Noted	Not Noted	Not Noted
Replicate Eggs	6 eggs	6 eggs	4 eggs	3 eggs per concentration tested	Not Noted
Number of Replicate Experiments	Not Noted	Not Noted	Not Noted	Not Noted	Not Noted
Endpoints Assessed	Hemorrhage, lysis, coagulation	Hemorrhage, lysis, coagulation	Hyperemia, hemorrhage, coagulation	Hemorrhage, lysis, coagulation	Dilation, hemorrhage, coagulation
Endpoint Evaluation	Endpoints scored from 0 (no reaction) to 3 (strong reaction)	The starting second that each of the endpoints is observed is recorded	Not Noted	The starting second that each of the endpoints is observed is recorded (see Kalweit 1990)	Numerical time-dependent scores for three endpoints.

	Balls et al. (1995) (Non-Transparent Substances)	Balls et al. (1995) (Transparent Substances)	Kojima et al. (1995)	Gettings et al. (1996) (HET-CAM I, II)	Gettings et al. (1996) (HET-CAM III)
Analysis Method	Calculation of "S Score". "S Score" is calculated using the most sensitive endpoint (endpoint can change from chemical to chemical). The scores recorded for the most sensitive endpoint are summarized for the 6 eggs. (Maximum for 6 eggs is 18)	Computer program calculates an Irritation Index. Irritation Index Is used to calculate a "Q Score". "Q Score" is a comparison of the Irritation Index of a test chemical with that of the reference chemical. If the effect of test chemical is identical to reference, Q is 1.0. If effect of test chemical is less irritating, Q is lower. If effect of test chemical is more irritating, Q is higher.	Score was calculated based on the time of onset for each endpoint. Mean value of 4 eggs calculated.	Mean Irritation Score (IS; determined at 10% concentration) of 3 eggs is calculated. The Irritation Threshold Concentration (ITC; lowest concentration producing a slight reaction during observation period) is calculated.	Time-dependent scores were used to calculate a single value (maximum of 21).
Classification Scheme	Non Irritation: $S < 6$; Moderately Irritating: $6 \leq S < 15$; Severely Irritating: $S \geq 15$	Non Irritating: $Q < 1.5$; Moderately Irritating: $1.5 < Q < 2.0$; Severely Irritating: $Q < 2.0$	Not Noted	Irritant (According to FHSA): $IS \geq 5.1$ or $IS/ITC \geq 3.0$	Irritant (According to FHSA): Score ≥ 4.83
GLP Compliance?	Yes	Yes	Not Noted	Not Noted	Not Noted
Notes			Cites Luepke (1985) and Luepke and Wallat (1985) as basis for scoring scheme and analysis method.		

	Gilleron et al. (1996)	Spielmann et al. (1996)	Gilleron et al. (1997)	Hagino et al. (1999)
Hen Strain	White Essex	Not Noted	White Essex	White Leghorn
Egg Criteria for Use	Eggs were 7 days old prior to start of incubation and weighed 60 ± 5 g	Not Noted	Eggs were 7 days old	Not Noted
Egg Storage (Prior to use)	Not Noted	Not Noted	Not Noted	Not Noted
Incubation Temperature (°C)	37.0 ± 0.5	Not Noted	37 ± 0.5	37.6
Relative Humidity (%)	62.5 ± 1.5	Not Noted	52.5 ± 2.5	about 70
Egg Rotation?	Yes, with large ends upward for 9 days	Not Noted	Not Noted	Yes, once an hour
Checking Egg Viability	Not Noted	Not Noted	Not Noted	Not Noted
Incubation Period	10 Days	Not Noted	10 Days	10 Days
Procedure for Opening Egg	Eggs were candled and non-viable eggs were discarded. The airspace delimited by the inner membrane at the large end of the egg was marked. The eggshell was removed using a dentist's rotating saw blade. The inner membrane was moistened with 1.5-2.0 mL of 0.9% NaCl and the egg was returned to the incubator (at 37) for a maximum of 20 mins. After incubation, the NaCl solution was removed, using a vacuum pump, and the inner membrane was removed with forceps.	Not Noted	Not Noted	Portion of the egg shell above the air space was removed.
Manipulation of CAM	A test substance applicator (TSA), which is comprised of a perlon mesh (pore diameter = 63 micron) locked between two Teflon rings, was placed on the CAM	Not Noted	A test substance applicator (TSA) was placed on the CAM	Drop of water is placed on the membrane (to avoid capillary bleeding). A silicone rubber ring is placed on the CAM.

	Gilleron et al. (1996)	Spielmann et al. (1996)	Gilleron et al. (1997)	Hagino et al. (1999)
Investigator Defined Test Substance Classes	Chemicals from various chemical classes	Chemicals from various chemical classes	Chemicals from various chemical classes	Surfactant, polyols, color additives, organic salts, PABA derivative, esters, inorganic chemicals, alcohols, amines, alkanolamines, carboxylic acids
Total Test Substances Evaluated	46	200	60	39
Test Substance Quantity or Volume	0.3 mL or 0.3 g of test substance placed inside the TSA	Not Noted	0.3 mL or 0.3 g of test substance placed inside the TSA	0.2 mL (placed inside the rubber ring on the CAM). Solids (0.2 g) are reduced to a fine powder with a No. 200 sieve and placed inside a rubber ring on the CAM.
Test Substance Concentrations Tested	Undiluted	1-100% solutions tested	Not Noted	0.1, 1, 10, and 100%
Application of Solids to CAM	Placed inside TSA	All tested substances appear to be solubilized	Placed inside TSA	Placed inside rubber ring
Preferred Solvent	0.9% NaCl	0.9% NaCl or olive oil	Not Noted	Not Noted
Rinse after Test Substance Application?	TSA (which contains the test substance) is removed after 20 seconds	Yes, after 5 minutes (for substances that were insoluble in water or oil and were colored).	TSA (which contains the test substance) is removed after 20 seconds	Yes, after 20 seconds with distilled water
Evaluation Period	Up to 300 seconds after test substance applied	Up to 300 seconds after test substance applied	Up to 300 seconds after test substance applied	At 0.5, 2, and 5 minutes after test substance applied
Controls and Test Standards	Positive Controls: benzalkonium chloride, dimethylformamide, imidazole	Not Noted	Not Noted	Not Noted
Number of Control Eggs	Not Noted	Not Noted	Not Noted	Not Noted
Replicate Eggs	3	Not Noted	Not Noted	4 eggs
Number of Replicate Experiments	3	Not Noted	Not Noted	Not Noted
Endpoints Assessed	Hemorrhage, lysis, coagulation	Hemorrhage, coagulation, lysis	Hemorrhage, lysis, coagulation	Hyperemia, hemorrhage, coagulation
Endpoint Evaluation	The starting second that each of the endpoints is observed is recorded	The starting second that each of the endpoints is observed is recorded	The starting second that each of the endpoints is observed is recorded	Numerical time-dependent scores for three endpoints.

	Gilleron et al. (1996)	Spielmann et al. (1996)	Gilleron et al. (1997)	Hagino et al. (1999)
Analysis Method	Irritation Index calculated using the formula: $(301\text{-sec H})/300*5+(301\text{-sec L})/300*7=(301\text{-sec C})^*9$; where H=Hemorrhage, L=Vessel Lysis; C=Coagulation; sec=starting second. Mean of 3 assays were calculated. Reproducibility also was assessed.	Irritation Score calculated ($(301\text{-sec H})/300*5+(301\text{-sec L})/300*7=(301\text{-sec C})^*9$; where H=Hemorrhage, L=Lysis; C=Coagulation; sec=starting second) for 10% solution calculated (IS10). Irritation Threshold (ITC; lowest concentration of a test substance to induce an irritant reaction on the CAM) calculated.	Irritation Index calculated using the formula: $(301\text{-sec H})/300*5+(301\text{-sec L})/300*7=(301\text{-sec C})^*9$; where H=Hemorrhage, L=Vessel Lysis; C=Coagulation; sec=starting second. Mean value and SEM of are calculated.	Not Noted
Classification Scheme	Non Irritant: 0-4.9; Irritant: 5.0-21	BGA Classification Model: Non/Slight: ITC > 10 and IS10 <16; Moderate: (1) ITC > 16 and IS10 > 16 or (2) ITC < 10 and IS10 < 16; Irritant (R36): (1) ITC < 10 and IS10 > 16 or (2) ITC < 2.5 and IS10 < 16; Severe (R41): (1) ITC ≤ 1% or (2) ITC between 1 and 2.5% and IS10 ≥ 16. Proposed model using mtc10 (see notes below): R41: mtc10 < 50 seconds.	Non Irritant: 0-4.9; Irritant: 5.0-21	Not Noted
GLP Compliance?	Not Noted	Yes	Not Noted	Not Noted
Notes		Cites Luepke (1985) as basis for protocol. Cites Kalweit (1990) and Spielmann (1991) as publishing the protocol used. Cites the standard protocol for the test method as InVitox Protocol.	Cites Gilleron 1996 as basis for protocol and analysis method	Cites Luepke 1985 as basis for scoring method
		Nine additional endpoints were conducted using the raw data and the IS and IT scores in this analysis. Of these the endpoints evaluated, that best correlated with in vivo classification was mtc10 (mean detection time for appearance of coagulation when using a 10% solution).		

Abbreviations: CAM = Chorioallantoic membrane, ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals, FHSA = Federal Hazardous Substances Act (1964), IS = Irritation score, IT = Irritation threshold concentration, NaCl = Sodium chloride, NaOH = Sodium hydroxide, SDS = Sodium dodecyl sulfate, Sec = seconds.